0026-895X/94/061210-07$03.00/0

MOLECULAR PHARMACOLOGY, 46:1210–1216, 1994.

Thallium Mediates a Rapid Chloride/Hydroxyl Ion Exchange Through Myelin Lipid Bilayers

RICARDO S. DÍAZ and JAIME MONREAL

Instituto Cajal, Consejo Superior de Investigaciones Científicas, E-28002 Madrid, Spain

Received June 15, 1994; Accepted September 21, 1994

SUMMARY

We have investigated the effects of several heavy metal cations on the proton and chloride permeabilities of liposomes prepared with endogenous lipids from brain myelin, by monitoring the fluorescence emitted by acridine orange and *N*-(6-methoxyquinolyl)acetoethyl ester. In addition to Hg^{2+} and Cu^{+}, nanomolar concentrations of Tl^{3+}, but not Tl^{+}, were able to generate a pH gradient (internally acidic) when an inwardly directed chloride gradient was established. No effect was observed either in the absence of Tl^{3+} or when Tl^{3+} was added (a) in the presence of chelating agents, reducing chemicals, or thiol compounds, (b) with identical intra- and extravesicular chloride concentrations, or (c) in the absence of chloride. Furthermore, Tl^{3+} was able to dissipate a pH gradient across the membrane for identical intra- and extravesicular chloride concentrations and to increase the chloride permeability in response to a pH gradient. All of these results suggest that Tl^{3+} behaves as a Cl^{-}/OH^{-} exchanger ionophore. Because the kinetics of the process did not vary with alterations of the membrane potential of the liposomes, it was concluded that the reaction is electroneutral, with a Cl^{-}/OH^{-} stoichiometry of 1:1. The results presented could explain some of the toxicological effects, largely unknown to date, of this extremely neurotoxic heavy metal and raise the possibility that thallium could have one of its main neurotoxicological targets in myelin.

Worldwide, thallium intoxication is considered the second most frequent cause of intended or accidental human poisoning (1). Thallium is a highly neurotoxic heavy metal, with a largely unknown mechanism of toxicity (2). Thallium has two oxidation states, Tl^{+} and Tl^{3+}. Both are protoplasmic toxins preferentially acting on the peripheral and central nervous systems, skin, gastrointestinal tract, cardiovascular system, and kidney (3). Sources of human exposure to thallium are mainly commercial rodenticides and human industrial activities, especially in coal-burning plants and cement plants (4).

Heavy metal toxicity is mainly explained by the interaction of these metals with cell proteins, such as metabolic enzymes, membrane channels, membrane transporters, etc. (5–7). The monovalent thallium cation Tl^{+} has been claimed to interact with cell enzymes such as pyruvate kinase, Na^{+}/K^{+}-ATPase, and aldehyde dehydrogenase (3). To explain some clinical and neuropathological effects of thallium poisoning, other interactions of this metal with cellular components have been suggested. Based on the similarities between chronic thiamine deficiency and thallium poisoning, an interaction between thallium and riboflavin has been postulated (8).

In contrast to most inorganic salts of heavy metals, hydrophobic derivatives such as the organometals trialkyl- and triphenyl-tin, triethyl-lead, and phenyl-mercury are able to cross the lipid matrix of biomembranes by diffusion, catalyzing an electroneutral Cl^{-}/OH^{-} exchange (9). One of the best studied cases is that of TET. *In vivo*, chronic or acute TET intoxication produces marked central nervous system demyelination, characterized by the splitting of myelin sheaths through the intraperiod lines and the generation of intramyelinic vacuoles (10–12). Such an effect is highly selective for myelin sheaths and appears to affect exclusively the lipid component of myelin membranes and not myelin proteins (10). Because thallium intoxication is frequently associated with primary and secondary demyelination (13, 14) and other inorganic cations of toxic heavy metals, such as Hg^{2+} and Cu^{+}, have been shown to catalyze an electroneutral Cl^{-}/OH^{-} exchange through lipid bilayers, as do organometals, we have studied the effects of the two oxidation states of thallium, Tl^{+} and Tl^{3+}, on the proton/hydroxyl and chloride permeabilities of liposomes prepared with endogenous myelin lipids. During the past few years model membranes, particularly liposomes, have been used as a pow-

This work has been supported by Grants PB93-0151 from Dirección Generalde Investigación Científica y Técnica to J.M. and a postdoctoral fellowship from Fundación Caja de Madrid to R.S.D.

ABBREVIATIONS: TET, triethyl-tin; Cl^{-}_{i}, intraliposomal chloride concentration; Cl^{-}_{o}, extraliposomal chloride concentration; K^{+}_{i}, intraliposomal potassium concentration; K^{+}_{o}, extralipsomal potassium concentration; MQAE, *N*-(6-methoxyquinolyl)acetoethyl ester; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; MES, 2-(*N*-morpholino)ethanesulfonic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DMSO, dimethylsulfoxide; LUV, large unilamellar vesicle; TBT, tributyl-tin; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

erful tool to study the general permeability properties of biological membranes, which closely resemble those of compositionally related lipid bilayers (15). We chose myelin lipids as liposome substrates because, in contrast to most other biomembrane lipids or lipid substrates, they offer an almost impermeable barrier to the diffusion of H^+/OH^- (16), representing a very suitable model to investigate the effects of those agents that modify the permeability of the membrane to H^+/OH^-. In addition, myelin lipids account for 75% of the dry weight of these membranes.

In this paper we report a novel molecular mechanism for thallium toxicity. Nanomolar concentrations of Tl^{3+}, but not Tl^+, catalyzed a rapid electroneutral Cl^-/OH^- exchange through myelin lipid bilayers. This mechanism could be of relevance for interpreting some of the clinical and neuropathological effects of thallium intoxication. The results are compared with the effects produced by Cu^+, Hg^{2+}, and the organometal TBT.

Experimental Procedures

Materials. The fluorescence probe MQAE was purchased from Molecular Probes. Nigericin, *N*-acetyl-L-cysteine, FCCP, acridine orange, MES, HEPES, tetramethylammonium hydroxide, EGTA, bovine serum albumin, choline chloride, potassium gluconate, and sodium gluconate were obtained from Sigma Chemical Co. Valinomycin was purchased from Boehringer Mannheim. Sodium thiosulfate, L-cysteine, EDTA, thallous acetate, thallic nitrate trihydrate, and thallic chloride were obtained from Merck. Thallic chloride tetrahydrate and 3,3′-dipropylthiadicarbocyanine iodide were from Aldrich. Solutions of thallic salts were prepared in either water, ethanol, or DMSO. Given the tendency of thallic salts to undergo hydrolysis, solutions in DMSO were found to be more stable and were used preferentially. Final concentrations of DMSO in the assay media were <0.1%. Most of the experiments shown in this paper were carried out with $TlCl_3 \cdot 4H_2O$ from Aldrich, but no differences were found when the other thallic salts were used. All other chemicals were of the highest grade available.

Lipids. Total myelin lipids, free of protein, were obtained by selective extraction of lyophilized brain white matter with tetrahydrofuran (17).

Buffers and liposomes. Unless otherwise stated, liposomes were prepared in 10 mM MES, 10 mM HEPES, adjusted to the indicated pH with tetramethylammonium hydroxide. The ionic composition and pH of the buffers were varied according to the requirements for each experiment and are described in the figure legends. LUVs were prepared from myelin lipids as described previously (17).

Fluorescence measurements. All fluorescence assays were performed at 25°, with continuous stirring, in a Shimadzu RF-5001 PC spectrofluorimeter, using a 3-ml quartz cuvette. A final volume of 2 ml of assay medium was used, and after each measurement both the stir bar and the cuvette were washed with 1 mM EDTA and rinsed extensively with deionized water. Changes in intraliposomal pH were monitored by using the ΔpH-sensitive probe acridine orange (18, 19). Being a weak base, acridine orange can move freely across membranes in its unprotonated form. If a pH gradient is established, acridine orange accumulates in its protonated form in the compartment where the pH is lower, in accordance with the pH gradient. Regional increases in the acridine orange concentration lead to dimerization of the dye and fluorescence quenching. Final concentrations of acridine orange and lipid in the assay medium were 1 μM and 0.28 mg/ml, respectively. Fluorescence was recorded at excitation and emission wavelengths of 492 and 530 nm, respectively. Excitation and emission slits were set at 1.5 nm and 3 nm, respectively. To calibrate the fluorescence signal of acridine orange in myelin lipid liposomes, varying outward potassium gradients were established across the liposomal membrane in the presence of the K^+/H^+ exchanger ionophore nigericin. Because the internal aqueous space trapped in the liposomes under the final conditions of the assays is negligible, compared with the total external volume, nigericin generates an outward transmembrane proton gradient of the same size as the initial potassium gradient (20). Higher K^+_i/K^+_o ratios produced greater quenching of the fluorescence of acridine orange, which shows a higher intraliposomal acidification. By plotting the percentage of fluorescence quenching versus the theoretical transmembrane pH gradient generated by nigericin (the logarithm of the initial K^+_i/K^+_o ratio), a straight line was obtained. This was used as a calibration line for equivalent experiments presented in this paper. Comparable results were obtained by using valinomycin plus FCCP instead of nigericin.

To detect changes in Cl^-_i, the Cl^--sensitive probe MQAE was used (21). MQAE fluorescence is strongly quenched by Cl^- by a collision mechanism. MQAE was added to the buffer used for LUV preparation at a final concentration of 5 mM. LUVs were then filtered through Sephadex G-25 minicolumns to remove the nontrapped fluorescent probe. Calibration was carried out with MQAE-containing LUVs prepared in chloride-free buffer (10 mM MES, 10 mM HEPES, 100 mM potassium gluconate, pH 7.4). Aliquots of the liposome suspension were diluted 70-fold into the same buffer in the presence of 5 μM nigericin and 10 μM TBT, and the fluorescence signal was recorded (F_i). Aliquots of a 1.5 M KCl stock solution were then added, and after each addition the fluorescence was recorded (F_e). In the presence of both nigericin and TBT, added KCl is readily equilibrated across the membrane, so Cl^-_i equals the final chloride concentration in the medium. By plotting F_i/F_e versus chloride concentration, a straight line was obtained. Fluorescence measurements were carried out at excitation and emission wavelengths of 355 and 460 nm, respectively. Slits were set at 3 nm (excitation) and 5 nm (emission).

Results

Because several organometals and some inorganic cations (i.e., Hg^{2+} and Cu^+) are able to catalyze a Cl^-/OH^- exchange in asolectin vesicles, we established an inwardly directed chloride gradient through myelin lipid vesicles (Fig. 1). Any substance able to catalyze that process should be able to generate a pH gradient by the efflux of OH^-, resulting in intraliposomal acidification and the quenching of acridine orange fluorescence. As expected, both TBT (Fig. 1B) and $HgCl_2$ (Fig. 1C) produced a concentration-dependent quenching of acridine orange. Interestingly, inorganic salts of trivalent thallium, i.e., thallic nitrate or thallic chloride, at nanomolar concentrations also provoked intraliposomal acidification (Fig. 1A). Such an effect was abolished when chloride was omitted, when Cl^-_i equaled Cl^-_o, or when salts of monovalent thallium (i.e., thallous acetate) were used instead of thallic salts (data not shown). These results strongly suggested that Tl^{3+}, but not Tl^+, could act as a Cl^-/OH^- exchanger ionophore through myelin lipid bilayers. On the other hand, cupric sulfate in the presence of 1 mM ascorbic acid (which ensures the complete reduction of Cu^{2+} to Cu^+) had a similar effect in the micromolar range of concentrations (data not shown). Other inorganic cations of heavy metals, such as Al^{3+} (in the forms of aluminic chloride or lactate), Ga^{3+} (gallic nitrate), or Cr^{3+} (chromium trichloride), were not effective, even at concentrations as high as 500 μM.

Tl^{3+} works at concentrations at least 10-fold lower, compared with Hg^{2+}, and, in addition, the Tl^{3+} kinetics for chloride-dependent ΔpH generation are faster. Such an effect is clearly observed in Fig. 1, *lower*, where the transmembrane pH gradient generated at two different times, 30 sec and 280 sec, after the addition of increasing concentrations of each compound is represented. In the case of Tl^{3+} (Fig. 1A, *lower*), the maximal

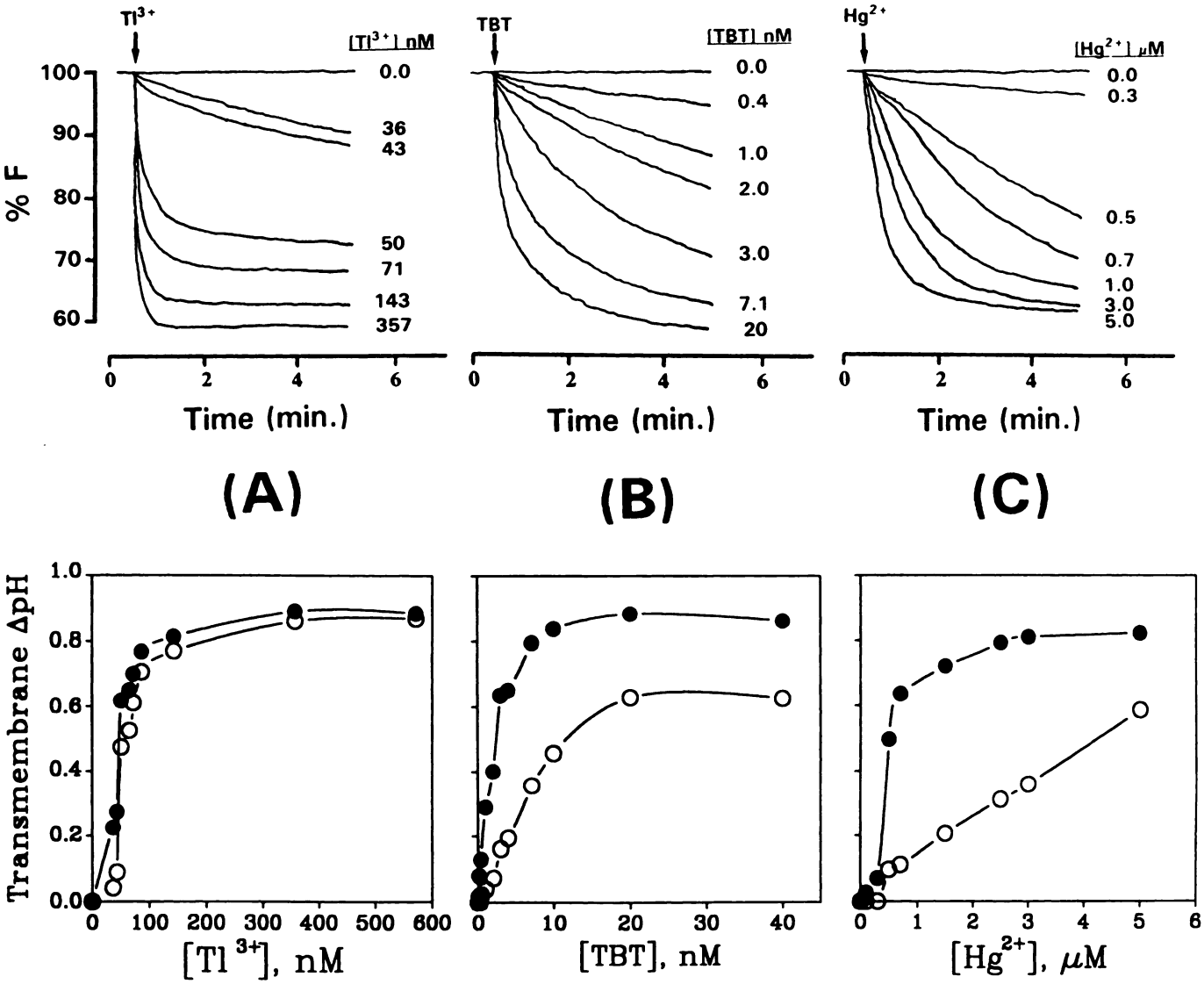

Fig. 1. Comparison of the effect of Tl^{3+} on the intraliposomal pH of LUVs with the effects of Hg^{2+} and TBT. *Upper*, LUVs prepared in 10 mM MES, 10 mM HEPES, 100 mM potassium gluconate, pH 7.4, were diluted 70-fold into the external medium (10 mM MES, 10 mM HEPES, 100 mM choline chloride, pH 7.4, 1 μM acridine orange). The addition of LUVs to the external medium did not result in any modification of the fluorescence signal. At the time indicated (*arrows*), increasing concentrations of either Tl^{3+} (A), TBT (B), or Hg^{2+} (C) were added. *Lower*, transmembrane pH gradients generated 30 sec (○) or 280 sec (●) after the addition of increasing concentrations of either Tl^{3+} (A), TBT (B), or Hg^{2+} (C) are shown.

effect was essentially achieved at 30 sec for every concentration, because the two curves almost overlap. This was not the case for Hg^{2+} (Fig. 1C, *lower*). TBT works at even lower concentrations than does Tl^{3+}, but with slower kinetics (Fig. 1B). The concentration needed to reach the half-maximal effect at 280 sec after the addition of each component (Fig. 1, *lower*) was 45 nM for Tl^{3+}, 470 nM for Hg^{2+}, 2.4 nM for TBT, and about 7 μM for Cu^{+}. Therefore, the relative potency of the compounds tested, in their capacity as Cl^{-}/OH^{-} exchangers, was TBT > Tl^{3+} > Hg^{2+} > Cu^{+}.

The effect of Tl^{3+} (200 nM final concentration) was completely suppressed by chelating agents (1 mM EDTA or EGTA), reducing chemicals (1 mM sodium thiosulfate or ascorbic acid), or compounds containing free sufhydryl groups (1 mM cysteine or *N*-acetyl-L-cysteine or 0.2 mg/ml bovine serum albumin) under the same experimental conditions as depicted in Fig. 1 (data not shown). Interestingly, EDTA or EGTA also suppressed the effect of Tl^{3+} in liposomes preincubated with this cation for 30 min at room temperature. Because this time is long enough for a metal-catalyzed lipid peroxidation process to take place, this result precludes the possibility that the intraliposomal acidification is mediated by the generation of hydroxyl radicals via a metal-catalyzed Fenton reaction.

Furthermore, the size of the transmembrane pH gradient generated by a same amount of Tl^{3+} depended markedly on the initial transmembrane chloride gradient. This is clearly shown in Fig. 2. Increasing inward chloride gradients produced increasing fluorescence quenching of acridine orange (Fig. 2A). When the transmembrane ΔpH generated at steady state was plotted versus Cl^{-}_{o}, a straight line was obtained (Fig. 2B). Other halides, such as bromides, were able to generate a similar transmembrane pH gradient in the presence of Tl^{3+} (Fig. 2B). Iodides could not be tested under the same conditions, because they nonspecifically quenched the acridine orange fluorescence.

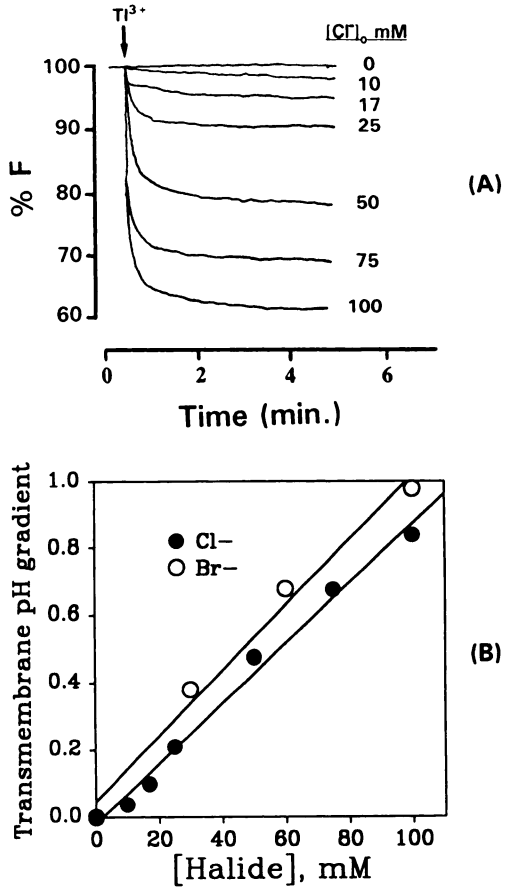

Fig. 2. Effect of transmembrane chloride gradient on Tl^{3+}-mediated intraliposomal acidification. A, LUVs prepared in 10 mM MES, 10 mM HEPES, 100 mM potassium gluconate, pH 7.4, were diluted 70-fold into the external medium composed of 10 mM MES, 10 mM HEPES, pH 7.4, and 1 μM acridine orange and containing choline chloride and potassium gluconate ([choline chloride] + [potassium gluconate] = 100 mM). At the time indicated (*arrow*), 200 nM $TlCl_3$ was added. B, A plot of the transmembrane pH gradient generated 280 sec after the addition of Tl^{3+} (from A) versus the extravesicular chloride concentration is shown (●). A straight line was obtained. Similar results were obtained by replacing chlorides with bromides (NaBr instead of choline chloride) (○).

In contrast to halides, oxyanions such as nitrate were ineffective (data not shown).

To further confirm the role of Tl^{3+} as a Cl^-/OH^- exchanger ionophore, we established a transmembrane ΔpH of 2 units across the membrane, for $Cl^-_i = Cl^-_o$. Under these experimental conditions, TBT and $HgCl_2$ were able to dissipate the initial pH gradient (data not shown). As depicted in Fig. 3, this was also the case for Tl^{3+}. Controls, lacking thallium, resulted in a stable base-line after the initial quenching, because myelin lipid liposomes exhibit negligible passive H^+/OH^- diffusion (16) (Fig. 3A). In contrast, concentrations as low as 25 nM Tl^{3+} resulted in a marked increase in the acridine orange fluorescence after the initial quenching, as OH^- entered liposomes and collapsed the established transmembrane pH gradient. The initial ΔpH dissipation rate was strongly dependent on thallium concentration. Such a dependence seems to be sigmoidal, as shown in Fig. 3B. In addition, for the same Tl^{3+} concentration, the kinetics of ΔpH dissipation were strongly stimulated by establishing a chloride gradient in the opposite direction from the OH^- gradient. Conversely, Tl^{3+} failed to dissipate a transmembrane pH gradient in the absence of Cl^- (data not shown).

Direct evidence for a Tl^{3+}-mediated chloride movement coupled to OH^- was obtained by using the halide-sensitive fluorescent probe MQAE (Fig. 4). An outwardly directed OH^- gradient and an inwardly directed chloride gradient were established simultaneously across the membrane of MQAE-containing LUVs. Any substance catalyzing the Cl^-/OH^- exchange should produce an influx of Cl^- at the expense of the efflux of OH^-. Indeed, the addition of Tl^{3+} resulted in a rapid quenching of the fluorescence, which reflects a rise in Cl^-_i (Fig. 4, *trace b*). MQAE fluorescence was not affected by Tl^{3+} in the absence of Cl^- (Fig. 4, *trace a*). The effect of Tl^{3+} was comparable to that of TBT under the same experimental conditions (data not shown).

In summary, all of these experiments suggested that Tl^{3+} is able to catalyze a Cl^-/OH^- exchange through the lipid bilayer. However, the process could take place by three different mechanisms. First, the process could have a stoichiometry of 1:1, being electroneutral. Second, the process, being a metal-catalyzed exchange, could be electrogenic, for example if Tl^{3+} promotes the exchange of two Cl^- ions for one OH^- ion. Third, Tl^{3+} could increase exclusively the Cl^- permeability of the membrane, generating an electrogenic Cl^- flux coupled to an electrophoretic OH^- counterflux. If the process is not electroneutral, then changes in the membrane potential or voltage clamping would alter the kinetics of the reaction. To determine which hypothesis better fits the mechanism of Tl^{3+}-mediated Cl^-/OH^- exchange, we performed the experiments depicted in Fig. 5. Chloride-containing liposomes (pH 6.0) were injected into a chloride-free buffer (pH 8.0) containing acridine orange, for $K^+_i = K^+_o$ (Fig. 5). The internally acidic pH gradient resulted in a rapid quenching of the fluorescence and a stable base-line. After the addition of 35 nM Tl^{3+} (final concentration), the rate of fluorescence recovery was recorded. Equivalent experiments were carried out for $K^+_i = K^+_o$ in the presence of the potassium ionophore valinomycin, which results in the clamping of the membrane potential near 0 (Fig. 5), and for $K^+_i > K^+_o$ in the presence of valinomycin, which generates, under these experimental conditions, an internally negative membrane potential of −118 mV, according to the Nernst equation (Fig. 5). As can be seen, the ΔpH dissipation rates were almost identical in the three experimental conditions. This supports the idea that the process catalyzed by Tl^{3+} is electroneutral. This conclusion was further confirmed in experiments carried out with the membrane potential-sensitive fluorescence probe 3,3′-dipropylthiadicarbocyanine iodide (data not shown).

Discussion

In this work we demonstrate that one of the two oxidation states of thallium, i.e., Tl^{3+}, behaves as an electroneutral Cl^-/OH^- exchanger ionophore when it is added to protein-free myelin lipid liposomes. Previous studies revealed the capacity of organometals (i.e., trialkyl-tins, triethyl-lead, and phenylmercuric acetate) to mediate a Cl^-/OH^- exchange through the lipid matrix of biomembranes as their major toxic mechanism. This finding has significant relevance, because the mechanisms of toxicity of thallium, in contrast to other toxic heavy metals, are scarcely understood (4). Two other inorganic cations of heavy metals, i.e., Hg^{2+} and Cu^+, were previously reported to have this effect in asolectin liposomes (9), and here we show that they retain this ability in myelin lipid liposomes (Fig. 1). However, in comparison with Hg^{2+}, Tl^{3+} works at 10-fold lower

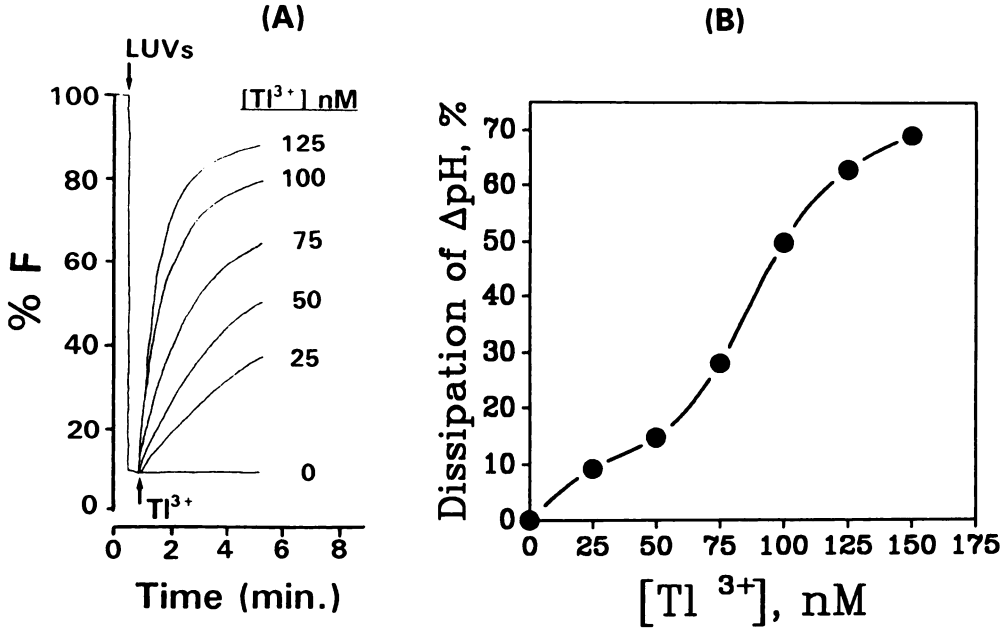

Fig. 3. Dissipation of a pH gradient by Tl^{3+}, for $Cl^-_i = Cl^-_o$. A, At the time indicated (*first arrow*), LUVs prepared in 10 mM MES, 10 mM HEPES, 100 mM choline chloride, pH 6.0, were diluted 70-fold into the external buffer (10 mM MES, 10 mM HEPES, 100 mM choline chloride, pH 8.0, 1 μM acridine orange). Increasing concentrations of $TlCl_3$ were then added (*second arrow*). B, The dissipation rate (50 sec) of the established pH gradient is plotted as a function of thallium concentration.

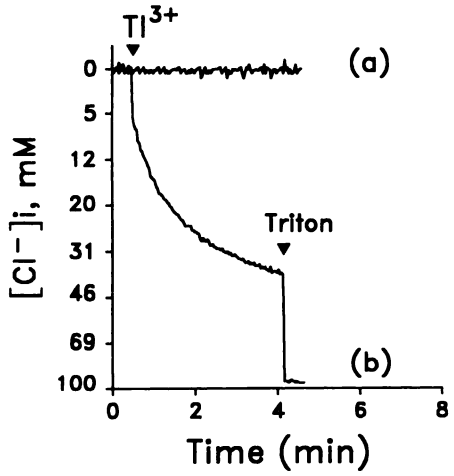

Fig. 4. Tl^{3+}-mediated chloride permeability in LUVs, measured using the Cl^--sensitive fluorescence probe MQAE. Liposomes were prepared in 10 mM MES, 10 mM HEPES, 100 mM potassium gluconate, 5 mM MQAE, pH 7.4. Nontrapped fluorescence probe was removed from the extravesicular space by gel filtration chromatography. MQAE-containing LUVs were diluted 70-fold into the external buffer of 10 mM MES, 10 mM HEPES, 100 mM potassium gluconate, pH 5.74 (*trace a*), or 10 mM MES, 10 mM HEPES, 100 mM KCl, pH 5.74 (*trace b*), and the fluorescence was recorded. Afterwards 40 μM $TlCl_3$ (final concentration) was added (*first arrowhead*). At the end of the experiment, liposomes were broken by the addition of 0.15% Triton X-100 (*second arrowhead*).

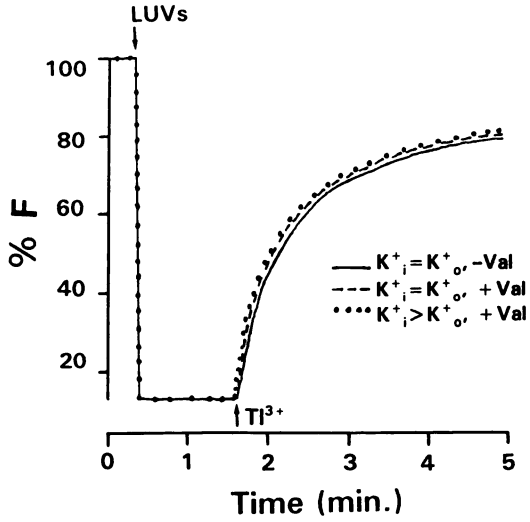

Fig. 5. Electroneutral nature of the Tl^{3+}-catalyzed Cl^-/OH^- exchange. LUVs were prepared in 10 mM MES, 10 mM HEPES, 100 mM KCl, pH 6.0. At the time indicated (*first arrow*), they were diluted 70-fold into the external medium containing 10 mM MES, 10 mM HEPES, 1 μM acridine orange, pH 8.0, and either 100 mM potassium gluconate ($K^+_i = K^+_o$, *−Val*), 100 mM potassium gluconate plus 0.6 μM valinomycin ($K^+_i = K^+_o$, *+Val*), or 100 mM sodium gluconate plus 0.6 μM valinomycin ($K^+_i > K^+_o$, *+Val*). Afterwards, 35 nM $TlCl_3$ was added in all cases (*second arrow*).

concentrations and the reaction is faster. The concentration of Tl^{3+} needed to reach the half-maximal effect is about 150-fold lower than the Cu^+ concentration (data not shown). TBT had the highest relative potency. Due to its strong hydrophobic nature, perhaps it diffuses more efficiently through the bilayer.

It is important to point out that the effect was restricted to the trivalent oxidation state of thallium. Neither thallous salts (i.e., thallous acetate) nor thallic salts in the presence of reducing agents (i.e., sodium thiosulfate or ascorbic acid) had any effect. This result was unexpected, because both forms are biologically active and highly toxic to mammals (22). This suggests that both salts could be converted *in vivo* to a common oxidation state, perhaps Tl^{3+}. Nevertheless, different biological effects of Tl^{3+} and Tl^+ should be expected.

Concerning the mechanism by which Tl^{3+} catalyzes the process, it is noteworthy that, at neutral pH, it is impossible to distinguish between H^+ and OH^- ion fluxes (23). This means that a process of Cl^-/OH^- exchange would be undistinguishable from Cl^-/H^+ cotransport, because the result would be the same, i.e., the generation of a pH gradient from a pre-established Cl^- gradient. Nevertheless, from the chemical point of view, Tl^{3+} is an acid ($pK_a = 1.15$) and forms hydroxyl species at neutral pH. On the other hand, because the process is electroneutral (Fig. 5) it is unlikely that this mechanism represents parallel conductive pathways for Cl^- and H^+. Furthermore, other acidic

heavy metals and organometals (Hg^{2+}, Cu^+, TBT, etc.,) have been shown to act via a mechanism similar to the proposed mechanism (electroneutral Cl^-/OH^- exchange). $TlCl_3$ is unstable in aqueous solutions, but it has a high solubility in organic solvents such as ethanol or DMSO. Thus, neutral $TlCl_3$ could be expected to cross the lipid matrix by diffusion. Indeed, previous papers have reported that chloride assists the diffusion of some heavy metals, such as Hg^{2+} (24) and Tl^+ (25), through lipid bilayers. In the experiments shown in Fig. 1, an inward Cl^- gradient exists through the bilayer, for identical intra- and extravesicular pH values. In the presence of relatively high Cl^-_o (100 mM), it is more likely that $TlCl_3$ would be nondissociated. Because of its hydrophobic character, it would diffuse across the lipid bilayer and would reach the internal Cl^--free side of the membrane and then become dissociated (producing Cl^-, $TlCl_2^+$, $TlCl^{2+}$, and Tl^{3+}). This step would assist the movement of Cl^- from the extraliposomal compartment to the intraliposomal compartment. Because the cationic forms of trivalent thallium are acidic, it would be expected that, in a Cl^--free environment, they would react with water, producing the hydroxyl forms $TlCl_2OH$, $TlCl(OH)_2$, and $Tl(OH)_3$. Assuming that these forms are hydrophobic enough to again cross the lipid bilayer from the chloride-free side to the chloride-rich side, they would liberate OH^- and close the cycle by again forming $TlCl_3$. The whole process would lead, therefore, to dissipation of the original Cl^- gradient and the generation of an internally acidic pH gradient.

Nevertheless, other selective interactions of Tl^{3+} with specific lipid components of liposomes cannot be ruled out. For example, $HgCl_2$ interacts specifically with phosphatidylethanolamine plasmalogen when incubated with whole myelin or extracted myelin lipids (26). This may explain why higher doses of $HgCl_2$ are needed to produce similar effects as a Cl^-/OH^- exchanger when myelin lipids (Fig. 1) are compared with asolectin (9). Although other lipid substrates have not been assayed in this work, factors such as bilayer thickness and asymmetric lipid distribution could probably influence the kinetics and the concentration of Tl^{3+} needed.

Concerning the biological consequences of thallium poisoning, little is known regarding molecular mechanisms. The monovalent thallium ion Tl^+ and K^+, having similar crystal ionic radii, can use the same pathways to enter the cells (i.e., potassium channels, Na^+/K^+-ATPase, or $Na^+/K^+/Cl^-$ cotransporters) (27). However, the crystal radius of trivalent thallium ion Tl^{3+} is very different from that of K^+. In addition, the potassium-like behavior of Tl^+ does not provide any obvious explanation for changes in the central or peripheral nervous systems (22). On the other hand, the *in vivo* interaction between thallium and riboflavin (8), leading to metabolic failure of the cells, is still conjectural. The results reported in this work suggest that thallium, in the form of Tl^{3+}, could lead to metabolic failure of cells mediating an electroneutral Cl^-/OH^- exchange through the lipid matrix of biomembranes. According to Mitchell's theory, oxidative phosphorylation (i.e., biosynthesis of ATP) is coupled in the mitochondria to a transmembrane ΔpH. Any substance able to dissipate this pH gradient would disturb oxidative phosphorylation. Indeed, well known Cl^-/OH^- exchangers such as TET are able to uncouple this process (28). Furthermore, subcellular distribution studies in rat tissues showed the presence in mitochondria of a substantial amount of thallium (22), which was able to uncouple oxidative phosphorylation (29).

Moreover, most cells maintain an inwardly directed Cl^- gradient across the plasma membrane. For instance, the extracellular chloride concentration in mammalian skeletal muscle cells is 120 mM, whereas the intracellular chloride concentration is approximately 4 mM. This means that Tl^{3+} should be able to acidify the cytoplasm in a manner similar to that depicted in Figs. 1 and 2. Intracellular acidification profoundly depresses *in vivo* cerebral respiration and impairs several ionic membrane transport systems and cell volume homeostasis. A substantial decrease of the intracellular pH may lead to cellular death.

The main reason myelin lipids were used as substrates in the present study is that they are H^+/OH^- impermeable (16). Another reason is that demyelination and axonal destruction are the dominant features in peripheral nerves examined late in the clinical course of thallium poisoning (22). Some reports have pointed out selective degeneration of myelin sheaths (13) or secondary demyelination as a consequence of axonal degeneration (14). The results in this paper suggest that either thallium-mediated intramyelinic edema similar to that observed *in vivo* in TET intoxication (11) or thallium-mediated metabolic failure of neurons followed by Wallerian degeneration of axons could contribute to these effects.

Acknowledgments

We thank Emilia Rubio for expert technical assistance. We also thank Dr. J. S. Aguilar for critical reading of the manuscript.

References

1. Prick, J. J. G. Thallium poisoning. *Handb. Clin. Neurol.* **36**:239–278 (1979).
2. Berman, E. Thallium, in *Toxic Metals and Their Analysis* (E. Berman, ed.). Heyden, London, 201–208 (1991).
3. Douglas, K. T., M. A. Bunni, and S. R. Baindur. Thallium in biochemistry. *Int. J. Biochem.* **22**:429–438 (1990).
4. Manzo, L., and E. Sabbioni. Thallium, in *Handbook on Toxicity of Inorganic Compounds* (H. G. Seiler and H. Sigel, eds.). Marcel Dekker, New York, 677–688 (1988).
5. Clarkson, T. W. Molecular and ionic mimicry of toxic metals. *Annu. Rev. Pharmacol. Toxicol.* **32**:545–571 (1993).
6. Shafer, T. J., M. L. Contreras, and W. D. Atchison. Characterization of interactions of methylmercury with Ca^{2+} channels in synaptosomes and pheochromocytoma cells: radiotracer flux and binding studies. *Mol. Pharmacol.* **38**:102–113 (1990).
7. Preston, G. M., J. S. Jung, W. B. Guggino, and P. Agre. The mercury-sensitive residue at cysteine 189 in the CHIP28 water channel. *J. Biol. Chem.* **268**:17–20 (1993).
8. Cavanagh, J. B. Peripheral nervous system toxicity: a morphological approach, in *Neurotoxicology* (K. Blum and L. Manzo, eds.). Marcel Dekker, New York, 1–44 (1985).
9. Karniski, L. P. Hg^{2+} and Cu^+ are ionophores, mediating Cl^-/OH^- exchange in liposomes and rabbit renal brush border membranes. *J. Biol. Chem.* **267**:19218–19225 (1992).
10. Kirschner, D. A., and V. S. Sapirstein. Triethyl tin-induced myelin oedema: an intermediate swelling state detected by X-ray diffraction. *J. Neurocytol.* **11**:559–569 (1982).
11. Yanagisawa, K., H. Ishigro, K. Kaneko, and T. Miyatake. Acetazolamide inhibits the recovery from triethyl tin intoxication: putative role of carbonic anhydrase in dehydratation of central myelin. *Neurochem. Res.* **15**:483–486 (1990).
12. Sapirstein, V. S., R. Durrie, C. E. Nolan, and N. Marks. Identification of membrane-bound carbonic anhydrase in white matter coated vesicles: the fate of carbonic anhydrase and other white matter coated vesicle proteins in triethyl tin-induced leukoencephalopathy. *J. Neurosci. Res.* **35**:83–91 (1993).
13. Bank, W. J., D. E. Plasure, K. Suzuki, M. Nigro, and R. Katz. Thallium poisoning. *Arch. Neurol.* **26**:456–464 (1972).
14. Davis, L. E., J. C. Standefer, M. Kornfeld, D. M. Abercrombie, and C. Butler. Acute thallium poisoning: toxicological and morphological studies of the nervous system. *Ann. Neurol.* **10**:38–44 (1981).
15. de Gier, J. Permeability barriers formed by membrane lipids. *Bioelectrochem. Bioenerg.* **27**:1–10 (1992).
16. Diaz, R. S., and J. Monreal. Unusual low proton permeability of liposomes prepared from the endogenous myelin lipids. *J. Neurochem.* **62**:2022–2029 (1994).

17. Díaz, R. S., J. Monreal, P. Regueiro, and M. Lucas. Preparation of a protein-free total brain white matter lipid fraction. *J. Neurosci. Res.* **31**:136–145 (1992).
18. Soleimani, M., G. A. Lesoine, J. A. Bergman, and P. S. Aronson. Cation specificity and modes of the $Na^+:CO_3^{2-}:HCO_3^-$ cotransporter in renal basolateral membrane vesicles. *J. Biol. Chem.* **266**:8706–8710 (1991).
19. Britten, C. J., R. G. Zhen, E. J. Kim, and P. A. Rea. Reconstitution of transport function of vacuolar H^+-translocating inorganic pyrophosphatase. *J. Biol. Chem.* **267**:21850–21855 (1992).
20. García, M. L., M. Kitada, H. C. Eisenstein, and T. A. Krulwich. Voltage-dependent proton fluxes in liposomes. *Biochim. Biophys. Acta* **766**:109–115 (1984).
21. Verkman, A. S., M. C. Sellers, A. C. Chao, T. Leung, and R. Ketcham. Synthesis and characterization of improved chloride-sensitive fluorescent indicators for biological applications. *Anal. Biochem.* **178**:355–361 (1989).
22. Manzo, L., and E. Sabbioni. Thallium toxicity and the nervous system, in *Metal Neurotoxicity* (S. C. Bondy and K. N. Prasad, eds.). CRC Press, Boca Raton, FL, 35–54 (1988).
23. Deamer, D. W., and J. Bramhall. Permeability of lipid bilayers to water and ionic solutes. *Chem. Phys. Lipids* **40**:167–188 (1986).
24. Gutknecht, J. Inorganic mercury (Hg^{2+}) transport through lipid bilayer membranes. *J. Membr. Biol.* **61**:61–66 (1981).
25. Gutknecht, J. Cadmium and thallous ion permeabilities through lipid bilayer membranes. *Biochim. Biophys. Acta* **735**:185–188 (1983).
26. Kirschner, D. A., and A. L. Ganner. Myelin labeled with mercuric chloride: asymmetric localization of phosphatidylethanolamine plasmalogen. *J. Mol. Biol.* **157**:635–658 (1982).
27. Skulskii, I. A., G. P. Gusev, A. O. Sherstobitov, and V. Manninen. Anion-dependent transport of thallous ions through human erythrocyte membrane. *J. Membr. Biol.* **130**:219–225 (1992).
28. Rose, M. S., and W. N. Aldridge. Oxidative phosphorylation, the effects of anions on the inhibition by TET of various mitochondrial functions and the relationship between this inhibition and binding triethyl tin. *Biochem. J.* **127**:51–59 (1972).
29. Melnick, R. L., L. G. Monti, and S. M. Motzkin. Uncoupling of mitochondrial oxidative phosphorylation by thallium. *Biochem. Biophys. Res. Commun.* **69**:68–73 (1976).

Send reprint requests to: Jaime Monreal, Instituto Cajal, Avenida del Doctor Arce 37, E-28002 Madrid, Spain.